Functional muscarinic receptors in cultured skeletal muscle

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We studied the influence of muscarinic and nicotinic stimulation on both phosphoinositide metabolism and intracellular calcium levels in rat skeletal muscle primary cultures. Both nicotine and muscarine induced an increase in cytosolic calcium measured by fluo 3 fluorescence in confocal microscopy. The mass of inositol (1,4,5)trisphosphate measured by radioreceptor assay rose 2- to 3.5-fold upon carbachol, nicotine, or muscarine stimulation. The muscarine effect was mimicked by oxotremorine-M; pirenzepine prevented the muscarine-induced inositol (1,4,5)trisphosphate increase, whereas 4- diphenylacetoxy-N-methyl piperidine methiodide was ineffective. A relatively small (40 fmol/mg protein) high-affinity 3-quinuclidinylbenzilate binding to rat myotube microsomes was consistent with the muscarinic effect found. On the other hand, the effect of nicotine on the mass of inositol (1,4,5)trisphosphate was totally suppressed in sodium-free medium. Expression of M1 muscarinic receptors coupled t